REVIEW

MTHFR 677C→T Polymorphism and Risk of Coronary Heart Disease

A Meta-analysis

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Context In observational studies, individuals with elevated levels of plasma homocysteine tend to have moderately increased risk of coronary heart disease (CHD). The *MTHFR* 677C→T polymorphism is a genetic alteration in an enzyme involved in folate metabolism that causes elevated homocysteine concentrations, but its relevance to risk of CHD is uncertain.

Objective To assess the relation of *MTHFR* 677C→T polymorphism and risk of CHD by conducting a meta-analysis of individual participant data from all case-control observational studies with data on this polymorphism and risk of CHD.

Data Sources Studies were identified by searches of the electronic literature (MEDLINE and Current Contents) for relevant reports published before June 2001 (using the search terms *MTHFR* and *coronary heart disease*), hand searches of reference lists of original studies and review articles (including meta-analyses) on this topic, and contact with investigators in the field.

Study Selection Studies were included if they had data on the *MTHFR* 677C→T genotype and a case-control design (retrospective or nested case-control) and involved CHD as an end point. Data were obtained from 40 (34 published and 6 unpublished) observational studies involving a total of 11 162 cases and 12 758 controls.

Data Extraction Data were collected on *MTHFR* 677C→T genotype, case-control status, and plasma levels of homocysteine, folate, and other cardiovascular risk factors. Data were checked for consistency with the published article or with information provided by the investigators and converted into a standard format for incorporation into a central database. Combined odds ratios (ORs) for the association between the *MTHFR* 677C→T polymorphism and CHD were assessed by logistic regression.

Data Synthesis Individuals with the *MTHFR* 677 TT genotype had a 16% (OR, 1.16; 95% confidence interval [CI], 1.05-1.28) higher odds of CHD compared with individuals with the CC genotype. There was significant heterogeneity between the results obtained in European populations (OR, 1.14; 95% CI, 1.01-1.28) compared with North American populations (OR, 0.87; 95% CI, 0.73-1.05), which might largely be explained by interaction between the *MTHFR* 677C→T polymorphism and folate status.

Conclusions Individuals with the *MTHFR* 677 TT genotype had a significantly higher risk of CHD, particularly in the setting of low folate status. These results support the hypothesis that impaired folate metabolism, resulting in high homocysteine levels, is causally related to increased risk of CHD.

JAMA. 2002;288:2023-2031 www.jama.com

HOMOCYSTEINE IS A SULFUR-containing amino acid that plays a pivotal role in methionine metabolism. Genetic defects of the enzymes or dietary deficiency of B-vitamin cofactors involved in this metabolism result in elevated homocysteine levels. Elevated homocysteine levels have been associated with increased risk of coronary heart disease (CHD),[1] but whether this association is causal is uncertain.[2] Observational studies have shown that individuals with low folate levels or intake have a higher risk of CHD,[3-6] and it is possible that these associations may be independent of homocysteine.[7]

A common polymorphism exists for the gene that encodes the methylene tetrahydrofolate reductase (*MTHFR*) enzyme, which converts 5,10-methylene tetrahydrofolate to 5-methyltetrahydrofolate, required for the conversion of homocysteine to methionine. Individuals who have a C-to-T substitution at base 677 of the gene (amino acid change

See also pp 2015 and 2042.

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A222V) have reduced enzyme activity and higher homocysteine[8] and lower folate levels than those without this substitution.[9-13] Elucidation of an association, if any, between this polymorphism and CHD risk might be informative regarding the hypothesis that impaired folate metabolism, resulting in high homocysteine concentrations, plays a causal role in the occurrence of CHD.

Individual studies and previous meta-analyses of such studies[8,14] included too few subjects to provide conclusive evidence for or against an association of this polymorphism and CHD risk.[15] The aim of this study was to assess the relation of the *MTHFR* 677C→T polymorphism with risk of CHD by conducting a meta-analysis of individual participant data from all case-control observational studies that had data on this polymorphism and risk of CHD.

METHODS

Data Sources and Study Selection

Eligible studies were identified by searching the electronic literature (MEDLINE and Current Contents) for relevant reports published before June 2001 (using the search terms *MTHFR* and *coronary heart disease*), by hand searching reference lists of original studies and review articles (including meta-analyses) on this topic, and by personal contact with investigators in the field. Studies were included if they had data on the *MTHFR* 677C→T genotype and a case-control design (retrospective or nested case-control) and involved CHD as an end point.

Among a total of 53 published studies that examined the relation between the *MTHFR* 677C→T polymorphism and CHD risk, 6 studies were not included because they did not have a proper case-control design[16-20] or they studied cardiovascular mortality[21] only. Data on 13 further studies were unavailable because the investigators were unable or unwilling to collaborate.[22-34] Data from 6 unpublished studies that fulfilled the eligibility criteria were included after personal contact with the investigators. Among the 6 unpublished studies, 4 had previously reported on the relationship between homocysteine and CHD,[35-38] whereas no data had been previously reported in 2 studies. Hence, data were available for these analyses from 40 studies (34 published[4,11,12,14,39-67] and 6 unpublished[35-38]) involving 11 162 cases and 12 758 controls (TABLE 1).

Data Extraction

Data were collected on *MTHFR* 677C→T genotype, case-control status, and plasma levels of homocysteine, folate, and other cardiovascular risk factors, if available. Data were checked for consistency with the published article or with information provided by the investigators and converted into a standard format for incorporation into a central database. In the majority of the studies that included cases with myocardial infarction, diagnosis was defined using World Health Organization criteria.[68] In most studies that included cases with coronary artery disease, diagnosis was based on angiographic confirmation of significant stenosis (≥50%) in at least 1 of the 3 major coronary arteries. However, 1 study also included cases with silent myocardial infarction and coronary revascularization.[4] If studies included both population-based controls and hospital-based controls, only data on population-based controls were included. All studies used a standardized method to determine *MTHFR* 677C→T genotype,[69] with 2 exceptions in which the method had been validated elsewhere.[70,71]

Data Synthesis

Assuming that a prolonged increase in plasma homocysteine of 1 µmol/L (0.14 mg/L) is associated with a 5% increase in CHD risk[1,72] and that the average homocysteine concentration is 2.5 µmol/L (0.34 mg/L) higher in TT-genotype patients than CC-genotype patients,[8] the expected odds ratio (OR) of CHD for the TT compared with the CC genotype would be about 1.13. With an average prevalence of the TT genotype of 12%,[8] more than 9526 cases and an equal number of controls were required to have sufficient statistical power to estimate an OR in the expected range, using a 2-sided α of .05 and 80% power.[72]

Plasma homocysteine and folate values were log-transformed to improve normality, and geometric means are shown. Differences between cases and controls and between *MTHFR* 677C→T genotypes were assessed using analysis of variance for continuous data and χ^2 tests for categorical data. We assessed whether the frequencies of CC, CT, and TT genotypes among controls in individual studies were consistent with the expected distribution (ie, in Hardy-Weinberg equilibrium) using the Pearson χ^2 test.

The OR and 95% confidence interval (CI) of CHD for the TT genotype or for the CT genotype compared with the CC genotype were assessed in each individual study using logistic regression. The analyses ignored matching of cases and controls on age and sex, which had been applied in some studies. The study-specific ORs were then pooled with adjustment for study. Possible heterogeneity between the results of individual studies or in groups defined by continent of origin or by study design was assessed using χ^2 tests.

To explore interaction between the *MTHFR* 677C→T genotype and folate status, 6 subgroups were created whereby folate status was defined as below or above the median serum/plasma folate level. Odds ratios were calculated for all subgroups, with the subgroup with CC genotype and high folate as the reference group.

Complete data on age, sex, smoking, hypertension, and hypercholesterolemia were only available in a subset of studies, and the possible effects of confounding by these risk factors on the relationship between *MTHFR* and CHD risk were assessed using multivariable logistic regression in this subset.

A funnel plot was created by plotting the OR of CHD for TT vs CC genotype against the number of individuals in each study. A pattern resembling a symmetrical inverted funnel implied absence of significant selection or publication bias. All analyses were

Table 1. Characteristics of Included Studies*

Source	Type of Study	Controls		
		Age, Mean (SD), y	Prevalence of TT Genotype, No. (%)	Homocysteine Level, Geometric Mean (95% CI), μmol/L†
Europe (22 Studies)				
Kozich et al, Czech Republic‡	Retrospective	47 (11)	59 (10.0)	9.7 (9.5-9.9)
Meleady et al, Europe‡	Retrospective	43 (10)	78 (10.8)	9.7 (9.5-9.9)
Meisel et al,[39] Germany, 2001	Retrospective	60 (10)	96 (9.7)	9.7 (9.5-10.0)
Abbate et al,[40] Italy, 1998	Retrospective	. . .	32 (30.2)	. . .
Ardissino et al,[41] Italy, 1999	Retrospective	42 (8)	37 (18.5)	. . .
Gemmati et al,[42] Italy, 1999	Retrospective	47 (13)	32 (16.0)	7.8 (7.2-8.2)
Girelli et al,[43] Italy, 1998	Retrospective	57 (13)	23 (16.8)	13.3 (12.6-14.3)
Kluijtmans et al,[14] Netherlands, 1997	Retrospective	. . .	106 (8.5)	. . .
Tanis et al, Netherlands‡	Retrospective	45 (8)	69 (9.0)	11.9 (11.6-12.2)
Verhoef et al,[44] Netherlands, 1997	Retrospective	50 (7)	7 (7.0)	11.8 (11.1-12.6)
Verhoeff et al,[45] Netherlands, 1998	Retrospective	. . .	38 (14.0)	. . .
Szczeklik et al,[46] Poland, 2001	Retrospective	41 (13)	15 (4.9)	10.8 (10.5-11.2)
Ferrer et al,[47] Portugal, 1998	Retrospective	57 (16)	5 (3.9)	. . .
Ferrer et al, Portugal‡	Retrospective	40 (13)	8 (16.0)	7.8 (7.2-8.7)
Fernandez-Arcas et al,[48] Spain, 1999	Retrospective	61 (16)	39 (18.3)	. . .
Thogersen et al,[49] Sweden, 2001	Prospective	54 (7)	7 (5.4)	11.5 (10.9-12.1)
Todesco et al,[50] Switzerland, 1999	Retrospective	57 (21)	28 (12.5)	10.3 (9.7-10.8)
Adams et al,[51] United Kingdom, 1996	Retrospective	57 (13)	29 (13.1)	. . .
Chambers et al,[52] United Kingdom (whites), 2000	Retrospective	50 (7)	41 (9.7)	10.2 (10.0-10.5)
Chambers et al,[52] United Kingdom (Indians), 2000	Retrospective	49 (6)	12 (3.2)	10.8 (10.5-11.1)
Fowkes et al,[53] United Kingdom, 2000	Prospective	63 (6)	21 (6.5)	. . .
McDowell et al,[54] United Kingdom, 1998	Retrospective	. . .	73 (12.1)	. . .
Subtotal		51 (13)	855 (10.2)	10.3 (10.2-10.4)
North America (10 Studies)				
Christensen et al,[55] Canada, 1997	Retrospective	42 (5)	13 (10.7)	8.6 (7.9-9.3)
Hopkins et al, United States‡	Retrospective	49 (6)	16 (10.9)	9.8 (9.4-10.3)
Ma et al,[11] United States, 1996	Prospective	60 (9)	39 (13.4)	10.2 (9.9-10.5)
Schwartz et al,[12] United States, 1997	Retrospective	38 (5)	47 (12.6)	10.6 (10.2-10.9)
Folsom et al,[47] United States, 1998	Prospective	56 (5)	47 (9.3)	8.9 (8.7-9.2)
Anderson et al,[56] United States, 1997	Retrospective	61 (12)	18 (12.4)	14.2 (13.3-15.2)
Malinow et al,[57] United States, 1997	Retrospective	61 (9)	11 (10.5)	8.8 (8.2-9.3)
Schmitz et al,[58] United States, 1996	Retrospective	59 (9)	27 (14.4)	9.3 (8.6-10.1)
Tsai et al,[59] United States, 1998	Retrospective	44 (11)	18 (11.5)	8.5 (8.0-8.9)
Verhoef et al,[60] United States, 1998	Prospective	59 (8)	72 (14.4)	. . .
Subtotal		53 (11)	308 (12.2)	9.8 (9.7-10.0)
Other Continents (8 Studies)				
Van Bockxmeer et al,[61] Australia, 1997	Retrospective	41 (6)	16 (11.4)	. . .
Silberberg et al, Australia‡	Retrospective	. . .	11 (9.8)	11.8 (11.4-12.3)
Morita et al,[62] Japan, 1997	Retrospective	48 (10)	79 (10.2)	. . .
Nakai et al,[63] Japan, 2000	Retrospective	60 (8)	22 (11.1)	. . .
Ou et al,[64] Japan, 1998	Retrospective	55 (6)	42 (13.6)	. . .
Inbal et al,[65] Israel, 1999	Retrospective	40 (5)	20 (10.7)	. . .
Gulec et al,[66] Turkey, 2001	Retrospective	37 (5)	5 (5.0)	. . .
Tokgozoglu et al,[67] Turkey, 1999	Retrospective	53 (10)	3 (5.3)	14.7 (12.6-17.3)
Total		51 (12)	1361 (10.7)	10.2 (10.1-10.3)

*Ellipses indicate data not available.
†To convert homocysteine from μmol/L to mg/L, divide by 7.397. CI indicates confidence interval.
‡Unpublished studies.

performed using SAS, version 6.12 (SAS Institute Inc, Cary, NC).

RESULTS

Characteristics of Included Studies

Table 1 shows the number of cases and controls and selected characteristics for the controls of included studies. About half the data came from studies involving European populations and about a quarter from those of North American populations. The age distribution was similar in all studies. The prevalence of TT genotype among controls varied considerably among studies, ranging from 3.2 (in UK Indians)[52] to 30.2 (in an Italian population).[42] The *MTHFR* 677C→T genotype frequencies in controls were in Hardy-Weinberg equilibrium in all but 3 studies.[53,54,60]

Characteristics of Cases and Controls by Genotype

TABLE 2 shows the geometric mean plasma concentrations of homocysteine and folate and the presence of established cardiovascular risk factors for cases and controls and within *MTHFR* 677C→T genotypes. Cases had a higher mean homocysteine concentration and a more adverse cardiovascular risk profile. There were no significant differences in plasma folate concentrations between cases and controls. Among both cases and controls, individuals with the TT and CT genotypes had higher plasma homocysteine concentrations and lower folate concentrations than individuals with the CC genotype. Among controls, individuals with the CT genotype had a lower body mass index and individuals with the TT genotype had lower creatinine concentrations compared with individuals with the CC genotype. Among cases, there were significant differences in the prevalence of male sex, hypercholesterolemia, and smoking among genotypes.

MTHFR 677C→T Polymorphism and Risk of CHD

FIGURE 1 shows the OR of CHD for the TT genotype compared with the CC genotype in individual studies and a summary estimate for the combined analysis of all studies with adjustment for study. Overall, individuals with the TT genotype had a significantly higher odds of CHD compared with individuals with the CC genotype (OR, 1.16; 95% CI, 1.05-1.28). There was a trend toward an increased risk for the CT genotype compared with the CC genotype (OR, 1.04; 95% CI, 0.98-1.10). There was significant heterogeneity among the results of individual studies ($\chi^2_{39}=63.8$; $P<.01$). The continent of origin appeared to account for most of this heterogeneity. Continent-specific ORs showed that CHD risk was significantly increased for individuals with the TT genotype compared with those with the CC genotype in Europe (OR, 1.14; 95% CI, 1.01-1.28) but not in North America (OR, 0.87; 95% CI, 0.73-1.05). There was no heterogeneity within European studies ($\chi^2_{21}=27.1$; $P=.17$) or North American studies ($\chi^2_9=4.2$; $P=.90$), but there was significant heterogeneity between the pooled estimates for Europe and North America ($\chi^2_1=6.6$; $P=.01$). Data on studies from other continents were too sparse to assess a continent-specific OR.

Table 2. Distribution of Homocysteine and Folate Levels and Prevalence of Known Cardiovascular Risk Factors for Cases and Controls and by Subgroup of the *MTHFR* 677C→T Genotype*

	Cases					Controls				
			MTHFR 677C→T Genotype					*MTHFR* 677C→T Genotype		
	No.	Overall	CC	CT	TT	No.	Overall	CC	CT	TT
MTHFR 677C→T genotype, %	11 162	. . .	44.3	43.4	12.3	12 758	. . .	46.4	42.9	10.7
Homocysteine, geometric mean (95% CI), μmol/L	6031	11.5 (11.4-11.6)	11.2 (11.0-11.3)	11.4 (11.3-11.6)†	13.4 (12.9-13.9)†‡	6720	10.2 (10.1-10.3)§	9.9 (9.8-10.0)	10.2 (10.1-10.3)†‡	11.4 (11.0-11.8)†‡
Folate, geometric mean (95% CI), nmol/L	3242	11.1 (10.8-11.4)	11.7 (11.2-12.1)	10.8 (10.4-11.2)†	9.8 (9.0-10.6)†‡	4472	11.2 (11.0-11.5)	11.7 (11.5-12.1)	11.0 (10.7-11.4)†‡	9.4 (8.7-10.1)†‡
Age, y	9004	56 (11)	56 (11)	56 (11)	56 (11)	10 383	51 (12)§	51 (12)	51 (12)	51 (13)
Body mass index‖	4062	26.7 (4.1)	26.7 (4.1)	26.7 (4.2)	26.6 (4.1)	4483	25.4 (3.8)§	25.6 (3.8)	25.3 (3.8)†	25.3 (3.8)
Creatinine, μmol/L	1788	88 (22)	88 (21)	88 (22)	89 (22)	2347	80 (16)§	80 (16)	80 (16)	78 (16)†
Sex, male, %	9630	82	84	81†	82	10 706	69§	70	69	69
Hypertension, %¶	7254	43	44	43	43	8364	19§	19	18	17
Hypercholesterolemia, %#	6510	29	31	29†	24†‡	8074	16§	16	17	14
Diabetes, %	6910	16	16	15	15	8144	5§	5	4	5
Current smoking, %	6477	39	38	39	42†	8036	27§	27	28	29
Current alcohol use, %	2576	67	66	68	72	3816	72§	71	73	71

*Data are given as mean (SD) unless otherwise noted. CI indicates confidence interval. To convert homocysteine from μmol/L to mg/L, divide by 7.397; to convert folate from nmol/L to ng/L, divide by 2.266; and to convert creatinine from μmol/L to mg/dL, divide by 88.4. Cases and controls were matched on age and sex in 9 studies,[39,41,47-49,59,60,67] on age only in 2 studies,[37,61] and on age and smoking in 1 study.[11] One study used frequency matching for age and sex[57] and 2 studies used frequency matching for age only.[12,38]
†$P<.05$ vs CC genotype.
‡$P<.05$ vs CT genotype.
§$P<.05$ vs cases.
‖Body mass index is calculated as weight in kilograms divided by the square of height in meters.
¶Hypertension was defined as systolic blood pressure of greater than 140 to 160 mm Hg and/or diastolic blood pressure of greater than 90 to 95 mm Hg and/or use of antihypertensive drugs.
#Hypercholesterolemia was defined as total cholesterol level of greater than 5.7 to 6.5 mmol/L (220-250 mg/dL) and/or use of lipid-lowering drugs.

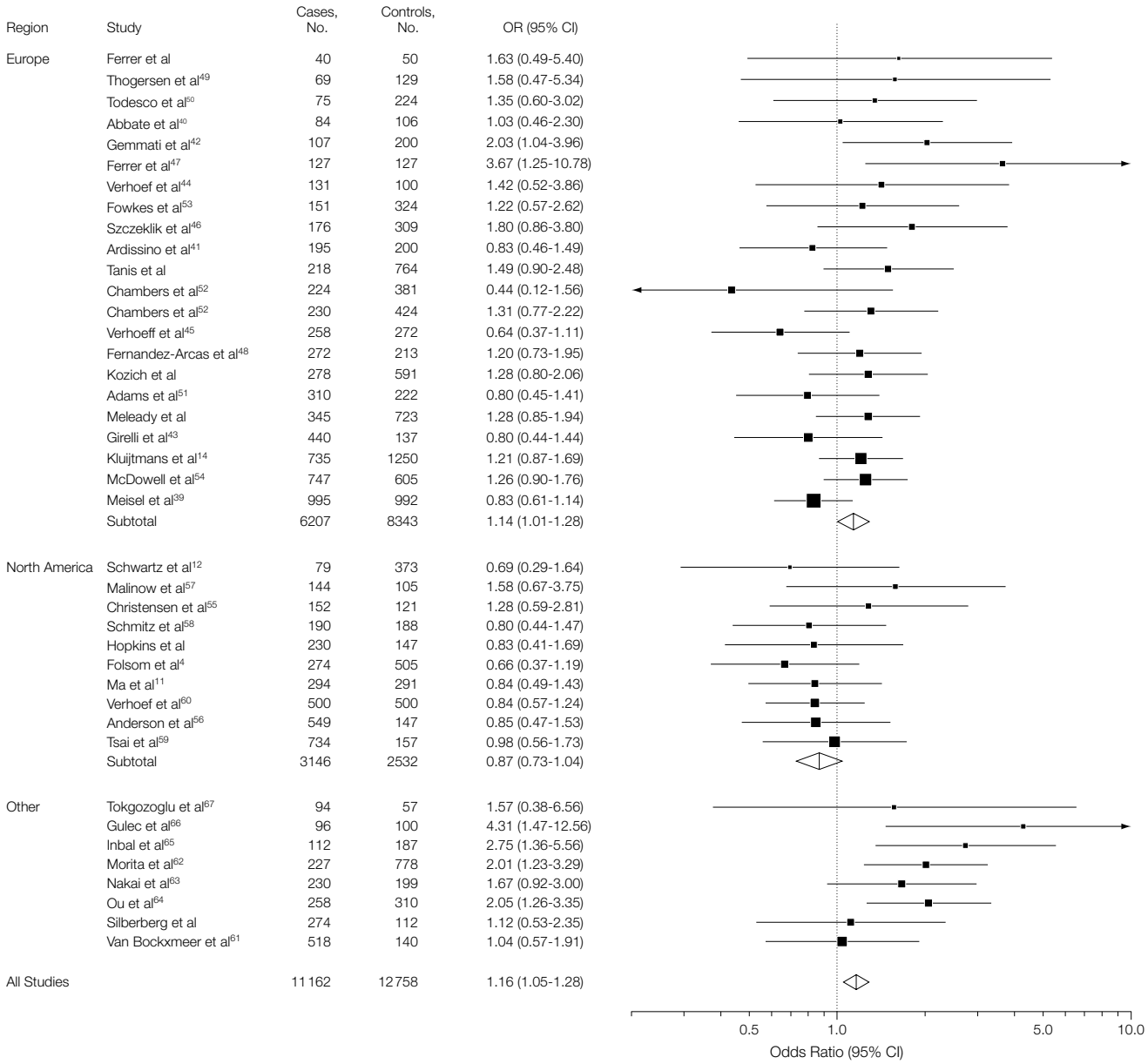
Effect Modification by Folate Status

The heterogeneity between European and North American studies may be explained by an interaction between *MTHFR* 677C→T polymorphism and folate status. TABLE 3 shows ORs of CHD within strata of the *MTHFR* 677C→T genotype and folate status for a subset of studies for which data on folate status was available. The results show that the TT genotype is associated with increased CHD risk only when folate status is low, which indicates an interaction between the *MTHFR* 677C→T polymorphism and folate status.

Prospective vs Retrospective Studies

To explore potential differences in the association between prospective and retrospective studies, we assessed pooled ORs for each study design. There was significant heterogeneity between the pooled estimates of prospective and ret-

Figure 1. Odds Ratios (ORs) and 95% Confidence Intervals (CIs) of Coronary Heart Disease for *MTHFR* 677 TT vs CC Genotype by Region of Origin



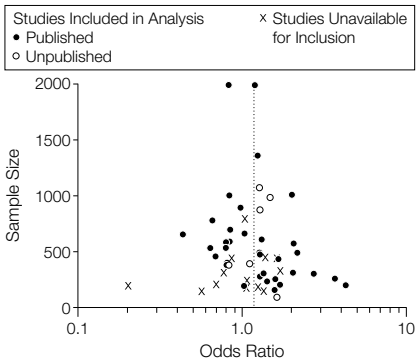
The size of the data markers is inversely proportional to the variance of the log ORs; horizontal lines represent the 95% CIs. Studies are ordered by the number of cases in each region. The combined ORs and the subtotals for each region and their 95% CIs are indicated by the diamonds.

Table 3. Odds Ratios (ORs) of Coronary Heart Disease (CHD) by Strata of the *MTHFR* 677C→T Polymorphism and Folate Status*

	MTHFR 677C→T Genotype		
	CC	CT	TT
Cases, No.	1543	1355	364
Controls, No.	2180	1847	445
Risk of CHD, OR (95% confidence interval)			
High folate status	1.00	0.91 (0.78-1.06)	0.99 (0.77-1.29)
Low folate status	1.24 (1.06-1.44)	1.32 (1.13-1.54)	1.44 (1.12-1.83)

*Folate status was defined as below or above the median serum or plasma folate level per continent. Data are based on references 4, 11, 12, 37, 38, 42-44, 46, 52, 54, 55, 57, 58, 61, 62, and 67, plus Ferrer et al and Hopkins et al (unpublished).

Figure 2. Funnel Plot of the Odds Ratios (ORs) of Coronary Heart Disease (CHD) for *MTHFR* TT vs CC Genotype for Each Study by Number of Individuals Studied



The plot shows the ORs for the 34 published and 6 unpublished studies and the 12 studies that were unavailable for inclusion in this analysis. Among the unavailable studies, 1 study[23] was omitted because the OR could not be abstracted and another study[29] was included twice because the data were presented separately in 2 different populations. The summary estimate of the OR of CHD for TT compared with CC is represented by a vertical dotted line.

rospective studies (χ^2_1=16.1; *P*<.001). The pooled OR of CHD for the TT genotype compared with the CC genotype was 0.86 (95% CI, 0.67-1.10) for prospective studies (5 studies involving 1288 cases and 1749 controls) and 1.21 (95% CI, 1.10-1.33) for retrospective studies (35 studies involving 9874 cases and 11 009 controls). However, since 3 of the 5 prospective studies were North American studies, it is likely that this subgroup analysis reflects a continent effect rather than an effect of the prospective study design.

Possible Confounding and Bias

The effect of confounding was explored in a subgroup of studies with available data on age, sex, smoking, hypertension, and hypercholesterolemia. Complete data on these cardiovascular risk factors were available for 5343 cases and 7308 controls. In this subgroup, the crude OR of CHD for the TT genotype vs the CC genotype was 1.15 (95% CI, 1.02-1.30). After adjustment for these confounding factors, the OR of CHD was 1.21 (95% CI, 1.06-1.38), thereby indicating that confounding is of little relevance to the overall results.

FIGURE 2 shows a funnel plot in which the OR of CHD for TT vs CC genotype was plotted against the number of individuals in each study. The figure includes data from published and unpublished studies and from studies for which data were not available. The shape of the funnel plot suggests that a few small studies finding an inverse association may not have been published. In addition, we calculated the average OR of CHD associated with TT compared with CC genotype among the 12 studies in which individual data were not provided for these analyses. The average OR for these studies was 1.15 (using the inverse of the variance as a weighting factor), suggesting that the present findings were probably not materially altered by the exclusion of studies for which data were unavailable.

COMMENT

Importance of the Genetic Association

This study involving 11 162 CHD cases and 12 758 controls from 40 studies demonstrated that individuals with the *MTHFR* 677 TT genotype have a 16% higher odds of CHD compared with individuals with the CC genotype. The results support the hypothesis that impaired folate metabolism, resulting in high homocysteine concentrations, plays a causal role in the occurrence of CHD. This meta-analysis illustrates the need to study a very large number of cases and controls to provide conclusive evidence for an association between genotype and disease in a setting in which the disease risk associated with a genotype is moderate.

Effect Modification by Folate and Other Factors

The *MTHFR* 677 TT genotype was significantly associated with a 14% increase in CHD risk in European populations but not in North American populations. Previous studies had shown that the *MTHFR* 677C→T polymorphism is only associated with high homocysteine levels or increased CHD risk in a setting of low folate status.[11,12,43,44,67,73,74] Hence, at higher dietary intakes of folate, the effect of the *MTHFR* 677C→T genotype has no adverse effect on plasma homocysteine levels or on subsequent risk of CHD. Our results confirm that a positive association between the *MTHFR* 677 TT genotype and CHD risk is mainly present when folate levels are low. However, we think that these results should be interpreted with caution since they are based on only part of the data and there might be misclassification of folate status because of the different assays used. Therefore, the absolute estimates might not be completely valid.

The average use of vitamin supplements has been consistently higher for several years in North America (25%-40%)[57,75-78] than in Europe (5%-15%).[35,79] While the North American studies were carried out before the enhancement of folate fortification in 1998, fortification of breakfast cereals had been introduced several years before this. Hence, it is very likely that effect modification by dietary intake of folate may account for at least some of the difference in the ORs of CHD obtained for the European and North American populations.[44,72,80] In the present study, com-

bined data from both cases and controls for each study showed that the mean homocysteine concentration was higher in European studies (10.9 µmol/L [1.47 mg/L]) than in North American studies (10.5 µmol/L [1.42 mg/L]). Moreover, the differences between *MTHFR* TT and CC genotypes were greater in European studies compared with North American studies for both homocysteine (2.1 vs 1.3 µmol/L [0.28 vs 0.18 mg/L]) and folate (2.5 vs 1.7 nmol/L [1.1 vs 0.75 ng/mL]) concentrations, respectively.

Additional sources of heterogeneity between Europe and North America may include effect modification by other cardiovascular risk factors[65,80-83] or linkage disequilibrium with other polymorphisms, such as the *MTHFR* 1298A→C polymorphism.[39,46,84] While the prevalence of hypercholesterolemia, smoking, and alcohol use was higher in European compared with North American studies (data not shown), these data were too sparse to examine possible effect modification by these factors.

Prospective vs Retrospective Studies

Studies on the association between the *MTHFR* 677C→T polymorphism and mortality or longevity have shown inconsistent results.[20,85-89] However, if individuals with TT have a higher case-fatality rate, then one might expect that the association in retrospective studies would be attenuated compared with that observed in prospective studies because retrospective studies are restricted to survivors, whereas prospective studies can include fatal and nonfatal outcomes. The present study showed that the TT genotype was associated with increased CHD risk in retrospective studies, but not in prospective studies, but this is likely to reflect differences in populations rather than an effect of prospective studies, considering that 3 of 5 prospective studies were North American studies.

Possible Influence of Bias

Although confounding is generally not anticipated in analyses of an association of a genotype with disease, there may be some imbalance in the distribution of cardiovascular risk factors by the *MTHFR* genotypes. Adjustment for the possible confounders in a subset of studies with available data did not attenuate the OR of CHD for the TT compared with CC genotype for *MTHFR*. However, the possibility of residual confounding cannot be completely excluded.

Another potential source of bias might be the inclusion of individuals from heterogeneous ethnic backgrounds. For example, the prevalence of the TT genotype is much lower in blacks (~1%) than in whites.[90] If the distribution of individuals with a specific ethnic background is unequal between cases and controls (so-called population stratification), this may bias an association between a genotype and risk of CHD. In a recent study, however, bias from population stratification in case-control studies was quantified and it was concluded that its impact is likely to be small, even if ethnicity is ignored.[91] Furthermore, the risk of population stratification in this meta-analysis is small since adjustment for study ensured that cases from each study were compared with their own controls.

It is unlikely that publication bias accounted for the results obtained; the funnel plot shows that only a few small negative studies may have been missed. Furthermore, selection bias is unlikely to have influenced the results, since the average OR of CHD associated with the TT genotype compared with the CC genotype of 12 studies that were unavailable for inclusion in these analyses was similar to our pooled OR.

Implications for Public Health

An accompanying article in this issue (see p 2015) describes a meta-analysis of 30 studies involving 5000 cases with ischemic heart disease, which showed that among prospective studies, a 25% lower usual homocysteine was associated with 11% (OR, 1.11; 95% CI, 1.04-1.17) lower risk of ischemic heart disease.[92] The concordance between the risk estimates obtained in these studies provides support for a causal association between homocysteine and CHD. Several large trials are currently under way to assess if homocysteine lowering by supplementation with folic acid and other B vitamins can reduce the risk of CHD.[93] Neither the meta-analyses nor these trials can solve the issue of whether high homocysteine levels per se or the accompanying low folate levels, which may operate via other mechanisms, are the cause of CHD. However, the present study provides some indirect evidence of the likely benefits of increasing population mean levels of folate, as the *MTHFR* genotype has no adverse effect on cardiovascular risk in the setting of normal folate status. Hence, provided that folate status is adequate, there is little clinical value of screening for *MTHFR* 677C→T genotype in the general population for prediction of CHD risk.

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Funding/Support: This work was financially supported by grants from the Dutch Organization for Scientific Research and the Wageningen Centre for Food Sciences. Henk Blom is an established investigator of the Netherlands Heart Foundation (D97.021).

Acknowledgment: We thank Sarah Lewington and Martijn Katan for their helpful comments on the protocol and manuscript and Paul Sherliker for graphic production.

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Obtained funding: Genest, Rozen, Fowkes, Girelli, Corrocher, Tokgozoglu, Rimm.
Administrative, technical, or material support: Samani, Ardissino, Merlini, van Bockxmeer, Folsom, Gulec, Hopkins, Kluijtmans, Jukema, Ashfield-Watt, Clark, Stangl, Gaziano, Szczeklik, Domagala, Tanis, Thogersen.
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